

Abstract

The aim of this thesis was to develop and optimize the packing of a new series of chromatographic columns called Astra with subsequent analytical measurements to determine the quality of optimized packing processes, the quality of the columns and to investigate the behavior of stationary phases.

The packing processes for the following columns were developed and optimized – Astra C18 HE, AQ and DM 5 μm (250/150/100 \times 4,6 mm, 150/100 \times 3,0 mm), Astra C18-HE 3 μm (250/150/100 \times 4,6 mm) , 150/100/50 \times 3,0 mm, 150/100/75/50 \times 2,1 mm), Astra AQ 3 μm (150/100 \times 4,6 mm, 150/100/50 \times 3,0 mm, 150/100/75/50 \times 2,1 mm), Astra DM 3 μm (150/100 \times 4,6 mm, 150/100 \times 3,0 mm, 150/100/50 \times 2,1 mm) and for zwitterionic columns 3 μm (150 \times 4,6 mm, 100 \times 2,1 mm). During the development of the packing processes, the following packing parameters were optimized. Composition of the suspension solution (slurry) – cyclohexanol:chloroform (1:10) for SF C18, AQ, DM and glycerin:methanol (1:3) for zwitterionic SF; concentration of the packing suspension; column pre-filling method – pre-filling with packing liquid (methanol); PID parameters, the values P = 3000, I = 100 and D = 100 were chosen; width of the pre-column, which must be equal to or greater than the inside diameter of the column; packing pressure and time; the column hardware type was chosen modular. At the same time, QC methods were developed to measure the standard Arion mix 3 in order to control the quality of these columns. Control values for the monitored QC parameters were introduced for selected columns and stability tests were performed. Furthermore, good repeatability of column packing has been confirmed. The relative standard deviations of the QC parameters of all analyte peaks in the standard Arion mix 1 ranged from 0.4 to 5.5%. The relative standard deviation of the SF mass in the packed columns was 0.57%.

Finally, the behavior of the stationary phases was investigated and tested. Different batches of stationary phases, the suitability of stationary phase DM was confirmed for some groups of substances, which are commonly determined by HPLC - amines, β -blockers and benzodiazepines (clonazepam and flunitrazepam), and finally the stationary phases were examined by Tanaka test, when high retention capacity for SF C18-HE and high ion exchange capacity and steric selectivity for SF DM was observed.

Key words: HPLC, packing of HPLC columns